partitioning of these three states depends on both force and the degree of supercoiling. However, the coexistence of these three states has not been described yet. Here we present a statistical physics model to describe DNA extension in the three coexisting states by calculating the full partition function. We compare these results to the extension of short DNA molecules at subpiconewton force in a range of linking number densities, measured with magnetic tweezers. Real-time analysis of the extension of short DNA tethers at constant force and linking number directly shows the dynamics of melting bubble formation, which we describe as a function of force and twist. Our results provide a comprehensive picture of the structure of underwound DNA and may have important consequences for various biological processes, in particular the ones that depend on local DNA melting, such as the initiation of replication and transcription.

149-Plat
Tuning DNA Bending with Charged Nanoparticles: Molecular Simulations
Abhishek Singh, Nan Li, **Yaroslava G. Yingling**.
North Carolina State University, Raleigh, NC, USA.
The interactions between DNA and charged nanoparticles have transpired into applications from biosensors to DNA-templated metallization. However, the process of non-specific DNA binding with NP is difficult to characterize and is not well understood. We performed molecular dynamics simulations to understand the mechanisms of DNA-NP interactions and observed that the ligand chemistry on the NPs has contrasting impact on the helical structure of DNA. All atom simulations show that uncharged NPs with -CH3 and -NH2 ligand end groups can only bind to the minor groove of DNA through hydrophobic interactions and do not induce a DNA helical distortion. On the other hand, charged NPs (from +6 to +60) can bind to both major and minor grooves of DNA. Moreover, while highly charged NPs wrap the DNA tightly, weakly charged NP can partially denaturate the DNA helix through a collective clustering behavior. Overall, we observed that by tuning the ligand chemistry and its density of GNP we can control the binding modes and the structural mechanics of DNA.

Platform: Protein Dynamics I

150-Plat
Conformational Analysis of Processivity Clamps Demonstrates that Protein Tertiary Structure Does Not Correlate with Dynamics
Penny J. Beuning, Jing Fang, Philip Nevin, John R. Engen.
Northeastern University, Boston, MA, USA.
Processivity clamps are critical for efficient DNA replication in all organisms. Whereas these clamp proteins can be either dimers or trimers, they all exhibit pseudo-six-fold symmetry. Each pseudo-monomeric domain is highly conserved in its tertiary structure even though the primary structures are divergent. We previously characterized the beta processivity clamp from *Escherichia coli* using hydrogen-deuterium exchange mass spectrometry (HXMS), which probes the solvent accessibility and hydrogen bonding of each backbone amide hydrogen, except those in prolines and at the first residue. We found that the three different domains within each monomer displayed different dynamics and that Domain I, which dissociates from Domain III to open the clamp, underwent partially cooperative local unfolding with a half-life of ~4 h. To determine how general our observations of a highly dynamic clamp protein were, we carried out a similar analysis using HXMS to characterize the dynamics of clamps from bacteriophage T4, the yeast *Saccharomyces cerevisiae*, archaeon *Thermococcus kodakarensis*, plant *Arabidopsis thaliana*, and human. *Thermococcus kodakarensis* and *Arabidopsis thaliana* both have two different PCNA clamp proteins that show slightly different dynamics. This is especially intriguing in the case of the two PCNA proteins from *Arabidopsis thaliana*, as the primary structures are 96% identical. The different clamps show a wide range of dynamic properties. Bacteriophage T4 gp45 shows high deuterium uptake and undergoes widespread local unfolding events with half-lives of approximately 5 min. In human PCNA, local unfolding is observed at the trimer interfaces with a half-life of about 1 h. The clamp proteins from bacteriophage T4, *Arabidopsis thaliana*, and human incorporate the most deuterium and are therefore the most dynamic. We find a striking range of dynamic properties of the clamp proteins despite their highly conserved tertiary structures.

151-Plat
FTIR and Femtosecond 2D-IR Spectroscopy of Azidohomoalanine-Labeled PDZ3 from PSD-95: Site-Specific Probing of Ultrafast Dynamics and Electrostatics in Proteins
Henrike M. Mueller-Werkmeister, Katharina Eberl, Martin Essig, Jens Bredenbeck.
Uni Frankfurt, Frankfurt, Germany.
Vibrational spectroscopy is a highly sensitive tool to study the structure and function of proteins. The absorption frequency of particular functional groups such as azides or nitriles can be sensitive to even small changes in the electrostatic environment. We use the methionine surrogate azidohomoalanine as a spectroscopic probe, which can be incorporated site-selectively during protein synthesis. Here we present recent work on the 3rd PDZ domain of PSD-95, a protein domain of 10.7kDa involved in protein-protein-interaction and signaling processes. The PDZ domain has various features that make it a suitable target for testing the application of an intrinsic azide group as a probe for the electrostatic environment. The protein displays both alpha-helical and beta-sheet secondary structure elements and has a hydrophobic peptide binding pocket. We incorporated azidohomoalanine at six positions in different secondary structures, in the interior and at the surface of the protein, as well as close to the binding pocket. Static IR spectra of all mutants show a clear correlation of the azide absorption frequency with the hydrophobicity of the surrounding side chains, the positions of which are known from x-ray structures. Furthermore changes in hydrophobicity upon ligand binding can be monitored, not only in FTIR data, but also in time-resolved 2D-IR spectroscopy. Using ultrafast 2D-IR spectroscopy we are able to measure fluctuations in the protein environment around our local probe on a picosecond timescale. Our data show that already on this timescale differences in dynamics upon ligand binding are induced.

152-Plat
Neutron Scattering Studies of Green Fluorescent Protein, Nanosecond-Picosecond Dynamics
Jonathan D. Nickels[1], Hugh M. O'Neill[2], Eugene Mamontov[2], Georg Ehlers[2], Madhusudan Tyagi[3], Alexei P. Sokolov[1].
[1]Oak Ridge National Lab/Univ. of Tennessee, Knoxville, Oak Ridge, TN, USA, [2]Oak Ridge National Lab, Oak Ridge, TN, USA, [3]National Institue for Standards and Technology, Gaithersburg, MD, USA.
We present a detailed analysis of the picosecond-to-nanosecond motions of green fluorescent protein (GFP) and its hydration water using incoherent and coherent neutron scattering and hydrogen/deuterium contrast. Our results reveal that while much of the dynamics in the hydrated protein are connected to motions of hydration water, there are significant differences in the dynamics of protein and its hydration water. On the picosecond-to-nanosecond timescale, the hydration water exhibits diffusive dynamics, while the protein motions are localized to less than ~3Å. The beta-barrel structure of GFP differs from previously studied globular proteins; which may explain the differences observed in the direct comparison of the atomic displacements (on a timescale of ~1ns) between GFP and lysozyme. We expand upon this and the recent Biophysical Journal article on this system with a more in depth analysis of the quasielastic spectra and a comparison of the data for GFP to current models for dry and hydrated protein motions. Finally, coherent scattering measurements allow us to comment on the cooperativity of protein and hydration water dynamics.

153-Plat
Co-Folding of the Histone Chaperone DAXX and H3.3/H4
Jamie E. DeNizio[1], Simon J. Elsässer[2], C. David Allis[3], Ben E. Black[1].
[1]University of Pennsylvania, Philadelphia, PA, USA, [2]MRC Laboratory of Molecular Biology, Cambridge, United Kingdom, [3]The Rockefeller University, New York, NY, USA.
Histone chaperones mediate the assembly and disassembly of canonical and variant nucleosomes. Currently there is little known of the conformational changes that occur upon chaperone-histone binding, but these are likely to be key to understanding the mechanism of histone transfer on and off of DNA. For this study, we focus on the death domain-associated protein (Daxx), a chaperone that specifically recognizes the H3.3 histone variant, mediating its deposition into heterochromatin. Using hydrogen/deuterium exchange (H/DX) coupled with mass spectroscopy to measure polypeptide backbone dynamics, we have obtained biochemical evidence of a co-folding mechanism of the Daxx histone-binding domain (HBD) with the H3.3/H4 histone dimer. Monomeric DAXX[HBD] undergoes extremely rapid H/DX, exhibiting the behavior of an unfolded protein. Upon binding to H3.3/H4, both Daxx and the H3.3/H4 subunits are globally stabilized, as measured by H/DX protection of several orders of magnitude. The H/DX rates in the ternary complex are matched at contact points between all three subunits as revealed by crystallography (Elsässer et al., 2012, in press), suggesting that they sample unfolded/folded states with the same kinetics throughout the complex. Importantly, Daxx binding stabilizes the helices of a H3.3/H4 complex in which point mutations disrupt stability in